# Green synthesis and characterization of zinc oxide and iron nanoparticles using various plants and evaluation of their biological potential.

Research protocol

For Registration in Ph.D. Program

Submitted by

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**Dated : ……**

# CERTIFICATE

This is to certify that this research protocol entitled **"Green synthesis and characterization of zinc oxide and iron nanoparticles using various plants and evaluation of their biological potential."** by **VARSHA GAUR**, submitted in fulfilment of the requirement for registration in Ph.D. Program in Department of Botany under School of Science, JECRC University, Jaipur, during the academic year 2023-2024, is a bonafide record work carried out under my guidance and supervision.

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## 1. Introduction

The subject of biology provides nanotechnology with models and components that serve as inspiration, as well as the essential tools and technical platform for analysing and manipulating living systems. Nanotechnology is a discipline which utilizes nanoscaled principles and technique to learn and manipulate biosystem, encompassing both living and non-living entities. This field utilizes both biological and physical principles to generate new devices and systems that are compacted with nanomaterials (Gericke and Pinches, 2006). Nanotechnology has become a crucial and quickly expanding subject of advanced research, with various uses in both electronics and healthcare (Glomm, 2005; Chan, 2006; Boisselier and Astruc, 2009). Nanotechnology joins different approaches to produce nano-scaled particles with advanced functions. Nanotechnology provides a more economical substitute for the conventional chemical and physical methods employed in nanoparticle production. The ways of formation can be categorized as intra-cellular and extracellular (Ahmad *et al*., 2003).

Nanoparticles can be synthesized using diverse methods, encompassing chemical, physical, and even biological processes. The synthesis of nanoparticles using chemical methods necessitated the utilization of potentially hazardous substances. Conversely, the synthesis of nanoparticles using physical techniques necessitated the use of elevated temperatures and substantial energy inputs, resulting in environmental repercussions (Ramos *et al*., 2020). Lately, there has been a heightened emphasis on utilizing green nanotechnology to manufacture nanoparticles that are non-toxic, environmentally friendly, and cost-effective. The constraints imposed by chemical and physical processes can potentially be overcome through "the application of green synthesis techniques, which involve the production of nanoparticles using naturally available sources such as plants and various microorganisms" (Bahrulolum *et al*., 2021).

The utilization of nanotechnology in drug delivery has been widely implemented to enhance the treatment efficacy of several disorders. Considerable attention has been directed towards the advancement of new nanoparticles and the characterization of their physicochemical attributes in connection to their biological behaviour and roles (Zhang *et al*., 2018).

Green nanotechnology utilizes "the principles of green chemistry and green engineering to create harmless and environmentally friendly nano assemblies that can address issues

impacting human health and the environment". The combination of green nanotechnology and drug delivery has given rise to a new field called "green nanomedicine" (Kanwar *et al*., 2019).

The utilization of plant extracts for the production of metal nanoparticles is a highly straightforward, convenient, cost-effective, and ecologically conscious approach that reduces the need for harmful chemicals (Vanlaveni *et al*., 2021). Due to their abundant availability, renewable nature, and environmentally benign characteristics, plant biomass is widely utilized in the synthesis of nanoparticles. Our group, as well as others, extensively focuses on using plant biomass as a catalyst for chemical synthesis (Rajkumari *et al*., 2019; Changmai *et al*., 2019).

Out of these options, ZnO-NPs are intriguing inorganic materials that offer multiple advantages. Zinc oxide nanoparticles (ZnO-NPs) have many applications in industries including energy conservation, textiles, electronics, healthcare, catalysis, cosmetics, semiconductors, and chemical sensing (Fortin *et al*., 2007; Naseem and Farrukh, 2015; Sheng-Nan *et al*., 2014; Siddiqui *et al*., 2013; Auffan *et al*., 2009). The nanoparticles (NPs) are safe and compatible with living organisms, and they have shown great potential in various medical applications. These include their ability to fight against cancer (Khodakovskaya *et al*., 2011), reduce inflammation (Allen *et al*., 2003), and kill harmful microorganisms. Additionally, they can be used to deliver drugs directly to specific targets in the body (Feizi *et al*., 2012), aid in wound healing, and assist in medical imaging (Ma *et al*., 2019; Luborsky, 1957).

Iron oxide nanoparticles demonstrate significant roles in the domains of biological science, including healthcare, agricultural, and the environmental science. Enhancing the non-toxic behavior and compatibility of magnetic nanoparticles (NPs) can be achieved by applying a specialized surface coating using either organic or inorganic molecules. These molecules can include "surfactants, medicines, proteins, starches, enzymes, antibodies, nucleotides, nonionic detergents, and polyelectrolytes". External magnetic fields can be employed to guide magnetic nanoparticles (NPs) towards certain organs, tissues, or tumours, facilitating hyperthermic treatment in patients (Ali *et al*., 2016).

The proposed study will primarily concentrate on the production and characterisation of zinc and iron nanoparticles. These nanoparticles will be produced utilizing extracts derived from three different plant species: *Acacia tortilis, Anogeissus pendula* Edgew, and *Ficus infectoria*.

The plants will be collected from Dhosi hill, which is on the boundary between Rajasthan and Haryana.

**About the selected plants**

***Acacia tortilis***

*Acacia tortilis* is a diminutive to moderate-sized tree that grows slowly and has a canopy shaped like an umbrella. It belongs to the Fabaceae family. The plant possesses both straight and curved thorns as a means of safeguarding its exceptionally nourishing leaves and pods from herbivorous animals. The blooms have a white coloration, possess a little size, emit a pleasant scent, and are arranged in compact groupings. Seeds are formed within pods that have a smooth and curved shape, and are arranged in a coiled structure resembling a spring. The plant exhibits a notable ability to endure drought, elevated temperatures, sandy and stony soils, as well as steeply inclined rooting surfaces. Furthermore, it has been noted that mature plants have frost resistance. The extracted oil exhibited a high viscosity and had a hue ranging from cream to light yellow. The total oil content was measured to be 3.8%. "The highest concentration of linolenic acid (50.43%) was observed in A. tortilis seed oil, followed by linoleic acid (36.75%), palmitic acid (6.41%), oleic acid (3.96%), and stearic acid (0.63%) (Heuze and Tran, 2015). It is commonly used in medical preparations to treat cough, cold, hoarseness, pharyngitis, gastric irritation and inflammation, diarrhea, dysentery, and pulmonary TB". It is also effective in soothing burns and scalds in the mouth (Yadav *et al*., 2013).

***Anogeissus pendula* Edgew**

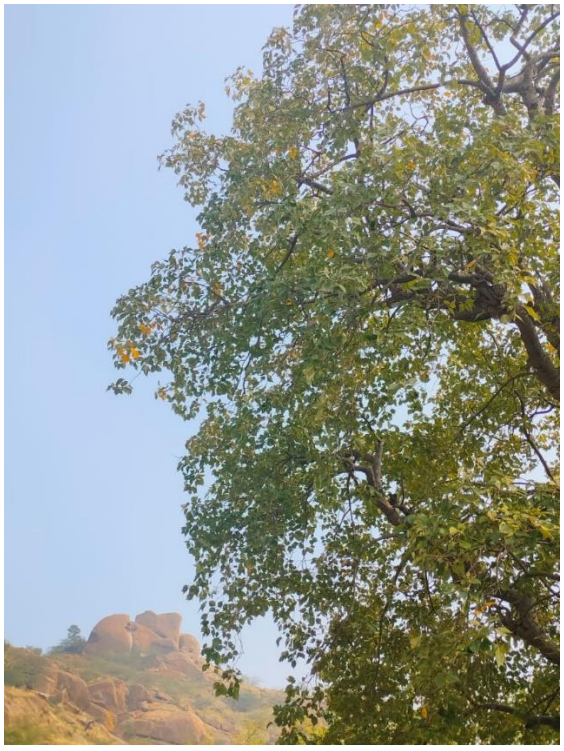
*Anogeissus pendula* Edgew, also known as 'dhok' or 'kardhii' in the local language, and sometimes referred to as the 'button tree' in English, belongs to the Combretaceae family. It is typically found in dry and mixed forests of India, where it grows as a clustered shrub or small tree. (Lata & Bhadoria, 2010; Scott, 1979). The stem bark, seed, fruit, and twig of the plant have been documented to have several ethnomedicinal applications, including treating stomach disorders, promoting wound healing, alleviating skin ailments, managing diarrhea and dysentery, relieving cough, and treating burns (Singh *et al*., 2016). Alkaloids and phenolic chemicals, including flavonoids, lignins, and tannins, have been found in seeds, leaves, and fruits (ArunaDevi *et al*., 2010; Hungund and Pathak, 1971; Raman *et al*., 2000). Phenolic chemicals are widely recognized for their strong antioxidant properties.

***Ficus infectoria***

*Ficus infectoria*, belonging to the Moraceae family, is a tree of moderate size, reaching a height of 10.0-12.0 meters and spreading in an 8.0-10.0 meter radius, with numerous branches. The leaves have a glossy green coloration and possess a substantial thickness of 10.0cm × 6.0cm. They are arranged alternately and have a thin shape. The blooms, on the other hand, are inconspicuous and white in color. The bark is 5 to 8 mm thick and has a flat appearance. The color of bark is brown, while the color of young leaves is reddish pink (Khare, 2007). Recent ethnopharmacological research indicates that *Ficus infectoria* is widely utilized throughout many regions for the treatment of several ailments, such as its antibacterial, antifungal, and hyperglycaemic effects in diabetic circumstances (Swami and Bisht, 1996; Chandira *et al*., 2010). Traditionally, bark decoctions are employed for cleansing ulcers, as a gargle to reduce excessive salivation, and for treating menstruation disorders and leucorrhoea. India, Bangladesh, Nepal, Pakistan, Sri Lanka, Southwest China, and Indochina are among the countries that have a rich history of using guava for traditional medical purposes.

***Acacia tortilis***

***Anogeissus pendula* Edgew**



*Ficus infectoria*

**Study site**

The hill is currently owned by three villages: 'Dhosi' in Jhunjhunu district, Rajasthan; and 'Thana' and 'Kultajpur' in Mahendergarh district, Haryana. The three settlements are situated near three waterfalls, which become active during the monsoon season in the months of July and August. These waterfalls originate from a water reservoir located at the summit of the hill. The eruption of Dhosi hill holds great reverence among Hindus. It is referenced multiple times in Sanskrit literature. This hill is renowned as the site where Chavan Rishi engaged in prolonged penance. A significant number of devotees attend the temple atop Dhosi Hill, which is dedicated to Chavan Rishi. Multiple fairs are held on the hill during different festivals and special occasions. The hill offers tourism prospects to diverse groups of tourists. For millennia, there has been an ancient sacred water reservoir on the hill. This reservoir was specifically built to preserve water for bathing purposes and to provide water for pilgrims. The rainwater accumulated in the sacred water reservoir possesses revitalizing powers and can be used for treating skin diseases. The water in the reservoir acquires herbal and 'Cupric' properties due to a significant presence of Copper metal in the surrounding hill and the abundant cultivation of unusual flora.

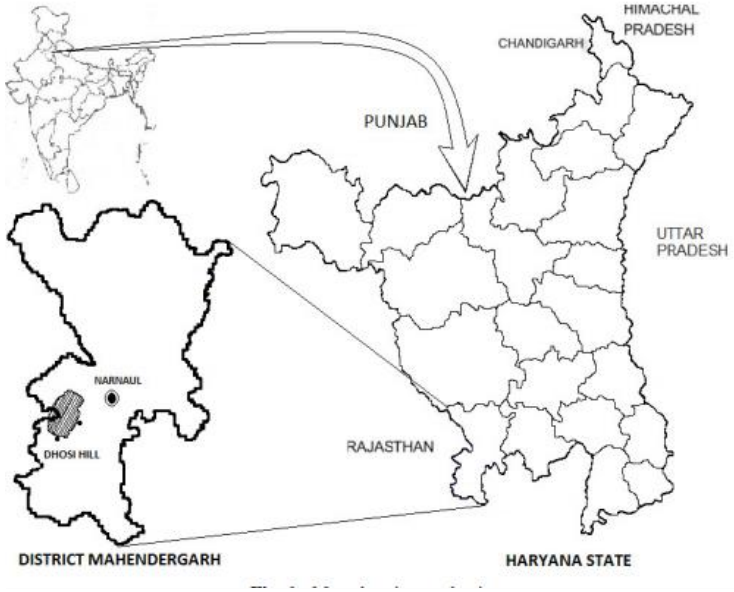


**Figure: The map showing Dhosi hill.**

**Figure: View of Dhosi Hill**

## 2. Review of literature

Nanomaterials (NMs) have become increasingly important in the 21st century due to their versatility and effectiveness in tackling worldwide research challenges in various fields like "energy, water and water sanitation, medicine, agriculture, material science, and the cosmetic industry". NMs, short for nanomaterials, are materials that have a dia ranging from 1 to 100 nm. Nanomaterials (NMs) have gained significance in technological improvement due to "their distinctive and better optical, mechanical, electrical, biological catalytic, and magnetic properties compared to their bulk form" (Janaki *et al*., 2015; Saleh, 2020). Nanomaterials possess distinct features that can be customized to match their intended use, thanks to their expansive surface area and dimensions. Moreover, the unique dimensions, configuration, and composition of NMs have a significant impact on their responsiveness, resilience, and other characteristics. These qualities render them suitable candidates for a diverse array of industrial and home applications, encompassing environmental, imaging, medical, energy, catalysis, cosmetics, and medicinal applications (Khan *et al*., 2019).

Some of the previous studies related to green synthesized zinc oxide and iron nanoparticles are given below-

**Zinc oxide nanoparticles**

Zinc oxide is a metal oxide that has been extensively studied because of its chemical stability, non-toxicity, cost-effectiveness, biocompatibility, and biodegradability (Agrawal *et al*., 2017; Weldegebrieal, 2020).

Plants, bacteria, yeast, and algae have effectively been utilized for the manufacture of ZnO nanoparticles, with plants demonstrating significant promise. This is because the process of synthesizing through plants may be easily scaled up for industrial production. It is a straightforward, environmentally friendly, cost-effective, and time-efficient method. Plants contain phytochemicals, such as "phenols, saponins, tannins, alkaloids, flavonoids, and carbohydrates". These compounds can be used as substitutes for toxic organic and inorganic chemicals, which are typically used as reducing or stabilizing agents in the traditional production of ZnO NPs (Akimtelu and Folorunso, 2020; Drummer *et al*., 2021).

The medicinal herbs Cassia fistula, Euphorbia hirta, Rosa indica, Costus pictus, and Solanum nigrum have been effectively used to create ZnO NPs, as reported by Suresh *et al*. (2015), Ahmad *et al.,* and Kalra (2020), Raj and Lawerence (2018), Suresh *et al*., (2018), and Ramesh *et al*., (2015) respectively. South Africa, located in the southern region of Africa, possesses a distinctive and varied array of plant species, some of which possess medicinal qualities. Additionally, a majority of these species are found exclusively in South Africa (Van *et al*., 2015). Nevertheless, there is a scarcity of reports regarding the utilization of indigenous medicinal herbs from southern Africa in the production of ZnO NPs. Consequently, more exploration of medicinal plants is necessary for the production of ZnO NPs.

*"Agathosma betulina, Plumbago auriculata, Lessertia montana, Lessertia frutescens, Tulbaghia violacea, Aspalathus linearis, Dovyalis caffra*, and *Athrixia phylicoides* DC are examples of indigenous medicinal plants from South Africa and southern Africa that have been studied for the production of ZnO nanoparticles". These synthesized nanoparticles have been investigated for various applications.

The content of phytochemicals in plant extracts differs depending on the extraction method, plant sections utilized, and specific plant species employed. Consequently, it is challenging to ascertain the mechanism behind the synthesis of ZnO NPs. Furthermore, due to the presence of several compounds in plant extracts, it poses a challenge to identify the specific components

accountable for the decrease and stabilization of NPs. Consequently, it becomes challenging to infer the process by which plants synthesize NPs. The synthesis process of nanoparticles is influenced by factors such as the phytochemical makeup, temperature, pH, reaction duration, and precursor concentration. These factors intrinsically impact both the quantity and quality of the synthesized nanoparticles (Jayachandran *et al*., 2021).

"The synthesized ZnO nanoparticles demonstrated strong antioxidant properties, as evidenced by their half maximal inhibitory concentration ($IC_{50}$) values of 120.31 µg/mL for 2,2-diphenyl-1-picrylhydrazyl (DPPH), 711.45 µg/mL for 2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ATBS), and 184.16 µg/mL for metal chelating. These values were compared to those of bulk ZnO and *L. montana* leaf extracts". Nevertheless, the quercetin control exhibited superior performance compared to the synthesized ZnO NPs in terms of ATBS and metal chelating. The synthesized ZnO nanoparticles shown potent antihyperglycemic activity, with $IC_{50}$ values of 0.0037 $gL^{-1}$ for α-glucosidase. In contrast, α-amylase showed a weaker inhibition with an inhibition of 0.620 $gL^{-1}$. The authors stated that the synthesized ZnO NPs exhibited a competitive mode of action on α-amylase, whereas their mechanism of action on α-glucosidase was non-competitive.

The antimicrobial efficacy was evaluated against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis*. The synthesized ZnO nanoparticles effectively suppressed the growth of all strains. However, they demonstrated exceptional antibacterial efficacy specifically against Gram-negative bacteria, achieving 95% inhibition against *E. coli* and 100% inhibition against *P. aureginosa*. In addition, the artificially produced ZnO nanoparticles demonstrated potent anticancer effects against A549 human lung cancer cells, resulting in a maximal cell death rate of 93.4% at a concentration of 1000 µg $mL^{-1}$.

Mbenga *et al*., (2022) effectively produced ZnO nanoparticles using an extract from *T. violacea* bulbs and examined the harmful effects of the synthesized nanoparticles. The ZnO nanoparticles that were created had a high level of crystallinity, adopting a hexagonal wurtzite phase. They were spherical in shape and densely arranged, with an average particle size of approximately 45.26 nm.

**Iron nanoparticles**

The green manufactured iron nanoparticles are derived from many plant sources, including "thyme (*Thymus vulgaris* (TV)), *Damask rose* (Rosa damascene (RD)), and stinging nettle (*Urtica dioica* (UD)) (Dykman and Khlebtsov, 2019). Iron nanoparticles were manufactured using green methods, utilizing plants such as common water-hyacinth (*Eichhornia crassipes*), lantana (*Lantana Camara*), and sensitive plants (*Mimosa pudica*). The plant produced iron nanoparticles with a standard size ranging from 20 to 60 nm. The nanoparticles have an uneven and aggregated quasi-spherical form (Wen *et al*., 2022). The green synthesis of iron nanoparticles significantly improved the physical, chemical, and biological characteristics of the nanoparticles (Garvasis *et al*., 2023). Iron nanoparticles generated from plant extracts possess antioxidant properties, as demonstrated by various processes such as the 2,2-diphenyl-1-picryl-hydroxyl (DPPH) radical scavenging assay, Folin-Ciocalteu method, and ferric reducing antioxidant power (FRAP)" (Wan *et al*., 2022). Phytochemical components such as polyphenols, flavonoids, and amino acids were crucial in the production of iron nanoparticles. The nanoparticles produced from phytochemicals have high efficacy in terms of antioxidant activity (Popoola, 2022).

The utilization of the green synthesis method in the production of zero-valent iron nanoparticles played a crucial role in the remediation of "brominated organic compounds, pesticides, azo dyes, alkaline-earth metals, malachite green, nitrate, monochlorobenzene, antibiotics, as well as the conversion of certain metals such as chromium, cobalt, and copper" (Lei *et al*., 2022). The rose apple (*Syzygium jambos* (L.) Alston) leaf extract, when mixed with water, was used to create zerovalent iron nanoparticles that effectively removed hexavalent chromium metal. The complete elimination of chromium was achieved by adjusting the amount of nanoparticles, controlling the temperature, and maintaining the appropriate pH level. The pH and concentration of nanomaterials play significant roles in the elimination of chromium metal (Constantinou *et al*., 2023). The coconut husk extract, when used to manufacture magnetite iron nanoparticles, had a limited capacity for absorbing calcium and cadmium (Pan *et al*., 2020). The leaf extract, when mixed with water at different ratios and temperatures, produced nanoparticles of zero-valent iron. The color changes seen in the nanosolution, along with the presence of phyto components, served as strong indicators of the reduction and stabilization processes involved in the synthesis of zero-valent iron nanoparticles (Wang *et al*., 2020). Zero-valent iron nanoparticles can be studied using methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and zeta potential analysis. The categorization analysis was validated based on the morphology, dimensions, and durability of

nanoparticles. The zero-valent iron nanoparticle effectively facilitated the removal of lead, with the time duration and concentration playing crucial roles. Specifically, a shorter duration was required for the removal of a smaller quantity of lead, while a higher concentration was necessary for the removal of a larger quantity of lead, albeit taking more time (Eljamal *et al*., 2020). The study highlighted the significant contribution of iron nanoparticles generated from citrus maxima peel extract in reducing waste and efficiently utilizing resources. A recent study highlighted the use of iron nanoparticles for the purification of water and soil. Specifically, zero-valent iron nanoparticles were found to possess highly effective catalytic characteristics (Harshiny *et al*., 2019).

The aqueous leaf extract of the Mediterranean cypress (*Cupressus sempervirens*) was used to produce iron nanoparticles, which shown significant efficacy in removing dye from wastewater. The effectiveness of this process was found to rely on both the duration of treatment and the concentration of the extract. The iron nanoparticle produced was employed for the purpose of eliminating methyl orange, achieving a 95% removal rate within a time frame of 6 hours (Ebrahiminezhad *et al*., 2017). Furthermore, an iron nanoparticle was produced using extracts from tea plant leaves (*Camellia sinensis*) and employed to reduce the pH indicator bromophenol blue. The researchers in the study conducted by Shahrashoub *et al*. (2021) utilized iron nanoparticles that were generated using green leaf extract for the purpose of degrading malachite green coloring. The study conducted by Wu *et al*., (2020) found that iron nanoparticles generated from the leaf extract of Eucalyptus globulus, often known as gum trees, showed significant potential for reducing eutrophic wastewater. The researchers in Selvaraj *et al*., (2022) employed iron nanoparticles produced from tangerine (*Citrus reticulate*) peel extract to eliminate cadmium from the water system. The utilization of the green synthesis method for producing iron nanoparticles has resulted in a decrease in chromium levels, with the extent of reduction depending on the concentration employed. Specifically, 1 mg of iron nanoparticle has been found to lower 500 mg of chromium (Banerjee *et al*., 2021).

Iron nanoparticles hold greater importance in the biomedical domain. The researchers in Sulaiman *et al*. (2018) used an extract from the flat-crown plant (*Albizia adianthifolia*) to create iron nanoparticles. These nanoparticles were then used to treat cancer cell lines MCF-7 and AMJ-13, leading to apoptosis. Furthermore, the iron nanoparticles produced from sugar apple (*Annona squamosa*) leaf extract exhibited a highly effective cytotoxic response against the cancer cell line (HepG2). The leaf extract of Babchi (*Psoralea corylifolia*) was used to

produce iron nanoparticles, which shown anticancer effects against renal carcinoma cell line (Caki-2 cells) in a study conducted by Rajendran *et al*. in 2021. The utilization of the green synthesis method in nanoparticle development held great significance in the treatment of human diseases (Gul *et al*., 2021).

## 3. Statement of the research problem/Research gap

Different kinds of metal nanoparticles synthesized using various plant extracts are being used in various sectors including healthcare industry. Among all metal nanoparticles zinc oxide nanoparticles and iron nanoparticles are of great interest as these have no toxic effect in limited quantity. There are various previous studies which reported potential of these nanoparticles for antimicrobial, antioxidant, antihyperglycemic, anti-inflammatory etc. activities.

The selected plants have not been used for synthesis of ZnONPs and FeNPs previously. There has been a lack of research for evaluating nanoparticles of the selected plants for their biological potential. So, in the proposed study, it will be the first attempt to synthesize, characterize and to evaluate zinc oxide and iron nanoparticles using extracts of the selected plants.

## 4. Objectives

The proposed study will be conducted to fulfil following objectives-

- To do Screening of different parts of the selected plants for different biological potentials.

- To synthesize zinc oxide and iron nanoparticles from extracts of the selected parts of *Acacia tortilis*, *Anogeissus pendula* Edgew. *Ficus infectoria,* and *Gnaphalium luteo* Album plants.

- To do characterization of the synthesized nanoparticles.

- To evaluate the synthesized nanoparticles for their antimicrobial and antioxidant potential.

- To analyse the synthesized nanoparticles for their anti-hyperglycemic potential.

## 5. Methodology

### Selection of plant parts

The above-mentioned plants will be collected from Dhosi hill. Different parts will be extracted in polar, mid polar and non-polar solvents and evaluated for their biological potential. Extracts showing maximum activities will be selected for further study.

### Synthesis of ZnO nanoparticles

In order to produce ZnO Nanoparticles, a solution of 0.02 M zinc nitrate hexahydrate will be created, consisting of 90 ml. This solution will then be subjected to heating at a controlled temperature of 30 °C ± 2 °C for a duration of around 10 minutes. The addition of plant extracts should be carried out gradually and with constant stirring. The solution will then be allowed to settle for a duration of 2 hours, during which the precipitate will accumulate at the bottom. The solution will subsequently undergo centrifugation for a duration of 10 minutes. The pellets will be gathered and subjected to a washing process using deionized water. The detection of zinc oxide nanoparticles can be inferred from the production of a precipitate with a creamy appearance. Subsequently, the final product was subjected to overnight drying in a hot air oven (Javed *et al*., 2022).

### Synthesis of iron nanoparticles

The procedure will be similar to ZnO. Instead of zinc nitrate iron (II) Sulphate will be used as source.

### Characterization of the synthesized green nanoparticles

The synthesized nanoparticles will be characterized for their size, shape, purity by –

- UV (Ultraviolet)
- FTIR (Fourier transmission infrared spectroscopy)
- FESEM (Field emission scanning electron microscopy)
- EDX (Energy dispersive X-ray spectrometer)

- XRD (X-ray diffraction)

**Evaluation of the antimicrobial activity**

Antimicrobial activity will be determined using well diffusion assay against some bacteria (Irobi *et al*., 1994) and fungi (Shafii *et al.*, 2005).

**Antioxidant activity**

Antioxidant activity will be determined using DPPH free radical assay (Valko *et al*., 2007) and FRAP assay (Wojtunik-Kulesza *et al*., 2020).

**Evaluation of anti-hyperglycemic activity**

Antidiabetic activity will be determined using glucose-DNSA assay (Miller, 1959).

**Statistical analysis**

All tests will be done in triplicates. Effects of nanoparticles will also be compared using plant extracts and standard drugs. Mean and standard deviation will be calculated. Using ANOVA, statistical variation in different doses of nanoparticles will be calculated using graph pad prism software.

**Instruments used**

Weighing balance, incubating shaker, magnetic stirrer, centrifuge, UV spectrophotometer, FTIR spectroscopy, FESEM, bacteriological incubator, laminar air flow, autoclave, vortex, sonicator, hot air oven, muffle furnace, refrigerated centrifuge etc.

**Details of External Resources**

For characterization of the synthesized nanoparticles, facilities of MRC, MNIT, Jaipur, and for evaluating biological activities, facilities of Biomitra Life Sciences Pvt. Ltd., Jaipur will be utilized.

## 6. Scope of the study

Mostly pathogenic bacteria and fungi are developing resistance for commercialized antibiotics which is a great concern for health. To control those harmful microbes, discovery of new effective, ecofriendly, and affordable drugs is needed. Similarly due to

increasing radiations and other pollutants, oxidative damage is also increasing day by day even in young people which cause requirement for more natural antioxidant compounds. Besides these, to avoid side effects caused by chemically synthesized antidiabetic drugs, more potential and safer drugs are required. Plant extracts have been proven for having such kinds of medicinal properties and in nanoform, metals do target specific and fast delivery of drug.

## 7. Month-wise work plan

The whole study is divided into 6 parts which will be proposed to be completed within 36 months approximately. The estimated month wise proposed plan in graphically represented as follows-

| **Work plan** | **Months** | | | | | |
|---|---|---|---|---|---|---|
| | **0-6** | **7-12** | **13-18** | **19-24** | **25-30** | **31-36** |
| Review of Literature | | | | | | |
| Collection of plant materials and selection of plant parts | | | | | | |
| Green synthesis and characterization of nanoparticles | | | | | | |
| Evaluation of plant extracts and green synthesized nanoparticles for antibacterial and antifungal | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| potential and data analysis | | | | | | |
| Evaluation of plant extracts and green synthesized nanoparticles for antioxidant and analysis of their data | | | | | | |
| Evaluation of plant extracts and green synthesized nanoparticles for anti-hyperglycaemic activity | | | | | | |
| Research paper and thesis writing | | | | | | |

## 8. Significance of the proposed study

Increasing contaminants in environment are causing increment in life threatening diseases. Chemically synthesized drugs are causing various side effects. The proposed study will pave a path for pharmaceutical industries to plan strategies for formulation of drugs alone or in combinations.

## 9. Limitations

As it will be in vitro study. The actual effects or side effects of the synthesized nanoparticles will be evaluated after study in vivo. So, this study will help only in proposing the new drugs for further study.

## 10. References


Adeyemi J.O., Elemike E.E., Onwudiwe D.C. ZnO Nanoparticles Mediated by Aqueous Extracts of Dovyalis Caffra Fruits and the Photocatalytic Evaluations. *Mater. Res. Express.* 2019;6:1–8. doi: 10.1088/2053-1591/ab5bcb. [CrossRef] [Google Scholar]

Adeyemi J.O., Onwudiwe D.C., Oyedeji A.O. Biogenic Synthesis of CuO, ZnO, and CuO–ZnO Nanoparticles Using Leaf Extracts of Dovyalis Caffra and Their Biological Properties. *Molecules.* 2022;27:3206. doi: 10.3390/molecules27103206. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Agarwal H., Venkat Kumar S., Rajeshkumar S. A Review on Green Synthesis of Zinc Oxide Nanoparticles—An Eco-Friendly Approach. *Resour. Effic. Technol.* 2017;3:406–413. doi: 10.1016/j.reffit.2017.03.002. [CrossRef] [Google Scholar]

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan ML, Kumar R, 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids and Surfaces B: Biointerfaces*. 28:313-8.

Ahmad J. Lipid Nanoparticles Based Cosmetics with Potential Application in Alleviating Skin Disorders. *Cosmetics.* 2021;8:84. doi: 10.3390/cosmetics8030084. [CrossRef] [Google Scholar]

Ahmad W., Kalra D. Green Synthesis, Characterization and Anti Microbial Activities of ZnO Nanoparticles Using Euphorbia Hirta Leaf Extract. *J. King Saud Univ. Sci.* 2020;32:2358–

Akintelu S.A., Folorunso A.S. A Review on Green Synthesis of Zinc Oxide Nanoparticles Using Plant Extracts and Its Biomedical Applications. *Bionanoscience.* 2020;10:848–863. doi: 10.1007/s12668-020-00774-6. [CrossRef] [Google Scholar]

Ali A, Zafar H, Zia M, Ul Haq I, Phull AR, Ali JS, Hussain A. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnol Sci Appl. 2016 Aug 19;9:49-67. doi: 10.2147/NSA.S99986. PMID: 27578966; PMCID: PMC4998023.

Allen M, Willits D, Mosolf J, Young M, Douglas T. Protein cage constrained synthesis of ferrimagnetic iron oxide nanoparticles. *Adv Mater Deerfield.* 2002;14(21):1562–1565. [Google Scholar]

ArunaDevi R, Lata S, Bhadoria BK, Ramteke VD, Kumar S, Sankar P, *et al*. Neuroprotective effect of 5,7,3',4',5'-pentahydroxy dihydroflavanol-3-O-(2''-O-galloyl)-beta-D-glucopyranoside, a polyphenolic compound in focal cerebral ischemia in rat. *Eur J Pharmacol.* 2010;626:205–12. [PubMed] [Google Scholar]

Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat Nanotechnol.* 2009;4(10):634–641. [PubMed] [Google Scholar]

B. Changmai , I. B. Laskar and L. Rokhum . Utilization of biowaste derived catalysts for biodiesel production: process optimization using response surface methodology and particle swarm optimization method. J. Taiwan Inst. Chem. Eng., 2019, **102** , 276 —282

Balogun F.O., Ashafa A.O.T. Potentials of Synthesised Lessertia Montana Zinc Oxide Nanoparticles on Free Radicals-Mediated Oxidative Stress and Carbohydrate- Hydrolysing Enzymes. *Acta Biol. Szeged.* 2021;64:239–249. doi: 10.14232/abs.2020.2.239-249. [CrossRef] [Google Scholar]

Banerjee S, Banerjee I, Dutta M, Pal R. Fabrication of iron nanoparticles using Leptolyngbya valderiana and investigation of its Cr (VI) removal potential in the free and biomass associated forms. *Algal Res.* 2021;58:102373. doi: 10.1016/j.algal.2021.102373. [CrossRef] [Google Scholar]

Birusanti AB, Espenti CS, and Mala S (2022). Plant-extract-assisted eco-friendly synthesis of iron oxide nanoparticles using cape gooseberry extract and their antibacterial study

Boisselier E, Astruc D, 2009. Gold nanoparticles in nanomedicine: preparation, imaging, diagnostics, therapies and toxicity. *Chem Soc Rev*. 38(6):1759-82.

C. P. Khare, figs and their medicinal value. "Indian Medicinal Plant" reprint. 2007, Springer, pp. 267. New Delhi.

Chan WCW, 2006. Bio nanotechnology progress and advances. *Biol Blood Marrow Tr*. 12:87-91.

Chandira R. M., Sahu C. M. and Jayakar B. “Antidiabetic activity of methanolic extract of bark of Ficus infectoria Roxb”. International Journal of Pharmacy & Life Sciences, 2010, vol. 1(5), pp. 278-281.

Constantinou D, Samanides CG, Koutsokeras L, Constantinides G, Vyrides I. Hydrogen generation by soluble CO2 reaction with zero-valent iron or scrap iron and the role of weak acids for controlling FeCO3 formation. *Sustain Energy Technol Assess.* 2023;56:103061. [Google Scholar]

Das S, and Patra CR (2021) Green synthesis of iron oxide nanoparticles using plant extracts and its biological application. In *Handbook of greener synthesis of nanomaterials and compounds* (pp. 139–170). Elsevier

Diallo A., Ngom B.D., Park E., Maaza M. Green Synthesis of ZnO Nanoparticles by Aspalathus Linearis: Structural & Optical Properties. *J. Alloy. Compd.* 2015;646:425–430. doi: 10.1016/j.jallcom.2015.05.242. [CrossRef] [Google Scholar]

Drummer S., Madzimbamuto T., Chowdhury M. Green Synthesis of Transition-Metal Nanoparticles and Their Oxides: A Review. *Materials.* 2021;14:2700. doi: 10.3390/ma14112700. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Dykman LA, Khlebtsov NG. Gold nanoparticles in chemo-, immuno-, and combined therapy: review. *Biomed Optics Exp.* 2019;10(7):3152–3182. doi: 10.1364/BOE.10.003152. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Ebrahiminezhad A, Taghizadeh S, Ghasemi Y, Berenjian A. Green synthesized nanoclusters of ultra-small zero valent iron nanoparticles as a novel dye removing material. *Sci Total Env.* 2018;621:1527–1532. doi: 10.1016/j.scitotenv.2017.10.076. [PubMed] [CrossRef] [Google Scholar]

Eljamal O, Thompson IP, Maamoun I, Shubair T, Eljamal K, Lueangwattanapong K, Sugihara Y. Investigating the design parameters for a permeable reactive barrier consisting of nanoscale zero-valent iron and bimetallic iron/copper for phosphate removal. *J Mol Liq.* 2020;299:112144. doi: 10.1016/j.molliq.2019.112144. [CrossRef] [Google Scholar]

Feizi H, Rezvani Moghaddam P, Shahtahmassebi N, Fotovat A. Impact of bulk and nanosized titanium dioxide (TiO2) on wheat seed germination and seedling growth. *Biol Trace Elem Res.* 2012;146(1):101–106. [PubMed] [Google Scholar]

Fortin JP, Wilhelm C, Servais J, Ménager C, Bacri JC, Gazeau F. Size-sorted anionic iron oxide nanomagnets as colloidal mediators for magnetic hyperthermia. *J Am Chem Soc*. 2007;129(9):2628–2635. [PubMed] [Google Scholar]

Freynman R.P. There's Plenty of Room at the Bottom. *Eng. Sci*. 1960;23:22–36. [Google Scholar]

Gericke M, Pinches A, 2006. Biological synthesis of metal nanoparticles. *Hydrometallurgy.* 83(1-4):132-40.

Glomm RW, 2005. Functionalized nanoparticles for application in bionanotechnology. *J Dispers Sci Technol*. 26(3):389-14.

Gul AR, Shaheen F, Rafique R, Bal J, Waseem S, Park TJ. Grass-mediated biogenic synthesis of silver nanoparticles and their drug delivery evaluation: a biocompatible anti-cancer therapy. *Chem Eng J*. 2021;407:127202. doi: 10.1016/j.cej.2020.127202. [CrossRef] [Google Scholar]

H. Bahrulolum, S. Nooraei, N. Javanshir, H. Tarrahimofrad, V.S. Mirbagheri, A.J. Easton, G. Ahmadian, 2021. Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. *J. Nanobiotechnol*.19: pp. 1-26

Harshiny M, AiswaryaDevi S, Matheswaran M. Spiny amaranth leaf extract mediated iron oxide nanoparticles: biocidal photocatalytic propensity, stability, dissolubility and reusability. *Biocatal Agric Biotechnol*. 2019;21:101296. doi: 10.1016/j.bcab.2019.101296. [CrossRef] [Google Scholar]

Heuzé V., Tran G., 2015. Umbrella thorn (Acacia tortilis). Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/339 Last updated on June 8, 2015, 14:39

Hoffmann M, Gerlach S, Hoffmann C, Richter N, Hersch N, Csiszár A, ... and Hoffmann B (2022) PEGylation and folic-acid functionalization of cationic lipoplexes—improved nucleic acid transfer into cancer cells. Front Bioeng Biotechnol 10 [PMC free article] [PubMed]

Huang X, Xu C, Ma J, Chen F. Ionothermal synthesis of Cu-doped Fe3O4 magnetic nanoparticles with enhanced peroxidase-like activity for organic wastewater treatment. *Adv Powder Technol*. 2018;29:796. doi: 10.1016/j.apt.2017.12.025. [CrossRef] [Google Scholar]

Hungund BL, Pathak CH. A survey of plants in Gujarat, India, for alkaloids, saponins and tannins. U.S.D.A. *Forest Service Research Paper*. 1971;NE-201:1–11. [Google Scholar]

Jamkhande P.G., Ghule N.W., Bamer A.H., Kalaskar M.G. Metal Nanoparticles Synthesis: An Overview on Methods of Preparation, Advantages and Disadvantages, and Applications. *J. Drug Deliv. Sci. Technol.* 2019;53:101174. doi: 10.1016/j.jddst.2019.101174. [CrossRef] [Google Scholar]

Janaki A.C., Sailatha E., Gunasekaran S. Synthesis, Characteristics and Antimicrobial Activity of ZnO Nanoparticles. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 2015;144:17–22. doi: 10.1016/j.saa.2015.02.041. [PubMed] [CrossRef] [Google Scholar]

K. Rajkumari , D. Das , G. Pathak and L. Rokhum , New J. Chem., 2019, **43** , 2134 —2140 RSC .

Kaningini A.G., Azizi S., Sintwa N., Mokalane K., Mohale K.C., Mudau F.N., Maaza M. Effect of Optimized Precursor Concentration, Temperature, and Doping on Optical Properties of ZnO Nanoparticles Synthesized via a Green Route Using Bush Tea ( Athrixia Phylicoides DC.) Leaf Extracts. *ACS Omega.* 2022;7:31658–31666. doi: 10.1021/acsomega.2c00530. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Kanwar R, Rathee J, Salunke DB, Mehta SK, 2019. Green nanotechnology-driven drug delivery assemblies. ACS Omega. 4(5): 8804-8815. https://doi.org/10.1021/acsomega.9b00304

Khan I., Saeed K., Khan I. Nanoparticles: Properties, Applications and Toxicities. *Arab. J. Chem.* 2019;12:908–931. doi: 10.1016/j.arabjc.2017.05.011. [CrossRef] [Google Scholar]

Khodakovskaya MV, de Silva K, Nedosekin DA, *et al*. Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions. *Proceedings of the National Academy of Sciences.* 2011;108(3):1028–1033. [PMC free article] [PubMed] [Google Scholar]

Lata S, Bhadoria BK. A novel chromone-substituted trimeric dihydroflavonol from *Anogeissus pendula*. *Chem Nat Compd.* 2010;46:726–9. [Google Scholar]

Lei M, Tang Y, Zhu L, and Tang H (2022) Chemical reductive technologies for the debromination of polybrominated diphenyl ethers: a review. J Environ Sci [PubMed]

Luborsky F. The kinetics of growth of spherical iron crystallites in mercury. *J Phys Chem.* 1957;61(10):1336–1340. [Google Scholar].

M.M. Ramos, E.D.S. Morais, I.D.S. Sena, A.L. Lima, F.R. De Oliveira, C.M. De Freitas, C.P. Fernandes, J.C.T. De Carvalho, I.M. Ferreira, 2020. Silver nanoparticle from whole cells of the fungi *Trichoderma* spp. isolated from Brazilian Amazon. *Biotechnol. Lett.,* 42. pp. 833-843

Ma Y, Kuang L, He X, *et al*. Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere.* 2010;78(3):273–279. [PubMed] [Google Scholar]

Mahlaule-Glory L.M., Mbita Z., Ntsendwana B., Mathipa M.M., Mketo N., Hintsho-Mbita N.C. ZnO Nanoparticles via Sutherlandia Frutescens Plant Extract: Physical and Biological Properties. *Mater. Res. Express.* 2019;6:085006. doi: 10.1088/2053-1591/ab1afa. [CrossRef] [Google Scholar]

Mauricio M.D., Guerra-Ojeda S., Marchio P., Valles S.L., Aldasoro M., Escribano-Lopez I., Herance J.R., Rocha M., Vila J.M., Victor V.M. Nanoparticles in Medicine: A Focus on Vascular Oxidative Stress. *Oxidative Med. Cell. Longev.* 2018;2018:1–20. doi: 10.1155/2018/6231482. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Mbenga Y., Mthiyane M.N., Botha T.L., Horn S., Pieters R., Wepener V., Onwudiwe D.C. Nanoarchitectonics of ZnO Nanoparticles Mediated by Extract of Tulbaghia Violacea and Their Cytotoxicity Evaluation. *J. Inorg. Organomet. Polym. Mater.* 2022;32:3249–3259. doi: 10.1007/s10904-022-02248-6. [CrossRef] [Google Scholar]

Melk M.M., El-Hawary S.S., Melek F.R., Saleh D.O., Ali O.M., el Raey M.A., Selim N.M. Nano Zinc Oxide Green-Synthesized from Plumbago Auriculata Lam. Alcoholic Extract. *Plants.* 2021;10:2447. doi: 10.3390/plants10112447. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Mungondori H.H., Ramujana S., Katwire D.M., Taziwa R.T. Synthesis of a Novel Visible Light Responsive γ-$Fe_2O_3$/$SiO_2$/C-$TiO_2$ Magnetic Nanocomposite for Water Treatment. *Water Sci. Technol.* 2018;78:2500–2510. doi: 10.2166/wst.2019.004. [PubMed] [CrossRef] [Google Scholar]

Naseem T, Farrukh MA. Antibacterial activity of green synthesis of iron nanoparticles using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract. *J Chem.* 2015;2015:7. [Google Scholar]

Ngoepe N.M., Mbita Z., Mathipa M., Mketo N., Ntsendwana B., Hintsho-Mbita N.C. Biogenic Synthesis of ZnO Nanoparticles Using Monsonia Burkeana for Use in Photocatalytic, Antibacterial and Anticancer Applications. *Ceram. Int.* 2018;44:16999–17006. doi: 10.1016/j.ceramint.2018.06.142. [CrossRef] [Google Scholar]

Ningthoujam R, Sahoo B, Ghosh P, Shivani A, Ganguli P, and Chaudhuri S (2023) Green production of zero-valent iron nanoparticles using pomegranate peel extracts and its use in lindane degradation. Nanotechnol Environ Eng 1–9

Ntozakhe L., Tichaona Taziwa R. *Zinc Oxide Based Nano Materials and Devices.* Intechopen; London, UK: 2019. Pyrolysis of Carbon-Doped ZnO Nanoparticles for Solar Cell Application. [Google Scholar]

Pan Z, Lin Y, Sarkar B, Owens G, Chen Z. Green synthesis of iron nanoparticles using red peanut skin extract: synthesis mechanism, characterization and effect of conditions on chromium removal. *J Colloid Interface Sci.* 2020;558:106–114. doi: 10.1016/j.jcis.2019.09.106. [PubMed] [CrossRef] [Google Scholar]

Raj A., Lawerence R. Green Synthesis and Charcterization of ZnO Nanoparticles from Leafs Extracts of Rosa Indica and Its Antibacterial Activity. *Rasayan J. Chem.* 2018;11:1339–1348. doi: 10.31788/RJC.2018.1132009. [CrossRef] [Google Scholar]

Rajendran A, Alsawalha M, Alomayri T. Biogenic synthesis of husked rice-shaped iron oxide nanoparticles using coconut pulp (Cocos nucifera L.) extract for photocatalytic degradation of Rhodamine B dye and their in vitro antibacterial and anticancer activity. *J Saudi Chem Soc.* 2021;25(9):101307. doi: 10.1016/j.jscs.2021.101307. [CrossRef] [Google Scholar]

Ramana DB, Singh S, Solanki KR, Negi AS. Nutritive evaluation of some nitrogen and non-nitrogen fixing multipurpose tree species. *Anim Feed Sci Technol.* 2000;88:103–11. [Google Scholar]

Ramesh M., Anbuvannan M., Viruthagiri G. Green Synthesis of ZnO Nanoparticles Using Solanum Nigrum Leaf Extract and Their Antibacterial Activity. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 2015;136:864–870. doi: 10.1016/j.saa.2014.09.105. [PubMed] [CrossRef] [Google Scholar]

Rastogi A., Tripathi D.K., Yadav S., Chauhan D.K., Živčák M., Ghorbanpour M., El-Sheery N.I., Brestic M. Application of Silicon Nanoparticles in Agriculture. *3 Biotech.* 2019;9:1–11. doi: 10.1007/s13205-019-1626-7. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Rethinasabapathy M, Kang SM, Haldorai Y, Jonna N, Jankiraman M, Lee GW, ... and Huh YS (2019) Quaternary PtRuFeCo nanoparticles supported N-doped graphene as an efficient bifunctional electrocatalyst for low-temperature fuel cells. J Ind Eng Chem 69:285–294

Saleh T.A. Nanomaterials: Classification, Properties, and Environmental Toxicities. *Environ. Technol. Innov.* 2020;20:1–11. doi: 10.1016/j.eti.2020.101067. [CrossRef] [Google Scholar]

Scott AJ. A revision of *Combretaceae*. *Kew Bull.* 1979;33:555–66. [Google Scholar]

Selvaraj R, Pai S, Vinayagam R, Varadavenkatesan T, Kumar PS, Duc PA, Rangasamy G. A recent update on green synthesized iron and iron oxide nanoparticles for environmental applications. *Chemosphere.* 2022;308:136331. doi: 10.1016/j.chemosphere.2022.136331. [PubMed] [CrossRef] [Google Scholar]

Shahrashoub M, Bakhtiari S, Afroosheh F, Googheri MS. Recovery of iron from direct reduction iron sludge and biosynthesis of magnetite nanoparticles using green tea extract. *Colloids Surf, A.* 2021;622:126675. doi: 10.1016/j.colsurfa.2021.126675. [CrossRef] [Google Scholar]

Sheng-Nan S, Chao W, Zan-Zan Z, *et al*. Magnetic iron oxide nanoparticles: synthesis and surface coating techniques for biomedical applications. *Chinese Phys B.* 2014;23(3):037503. [Google Scholar]

Siddiqui MH, Al-Whaibi MH, Sakran AM, *et al*. Calcium-induced amelioration of boron toxicity in radish. *J Plant Growth Regul.* 2013;32(1):61–71. [Google Scholar]

Singh D, Baghel US, Gautam A, Baghel DS, Yadav D, Malik J, *et al*. The genus *Anogeissus*: A review on ethnopharmacology, phytochemistry and pharmacology. *J Ethnopharmacol.* 2016;194:30–56. [PubMed] [Google Scholar]

Sulaiman GM, Tawfeeq AT, Naji AS. Biosynthesis, characterization of magnetic iron oxide nanoparticles and evaluations of the cytotoxicity and DNA damage of human breast carcinoma cell lines, Art cells. *Nanomed Biotechnol.* 2018;46(6):1215–1229. [PubMed] [Google Scholar]

Suresh D., Nethravathi P.C., Rajanaika H., Nagabhushana H., Sharma S.C. Green Synthesis of Multifunctional Zinc Oxide (ZnO) Nanoparticles Using Cassia Fistula Plant Extract and Their Photodegradative, Antioxidant and Antibacterial Activities. *Mater. Sci. Semicond. Process.* 2015;31:446–454. doi: 10.1016/j.mssp.2014.12.023. [CrossRef] [Google Scholar]

Suresh J., Pradheesh G., Alexramani V., Sundrarajan M., Hong S.I. Green Synthesis and Characterization of Zinc Oxide Nanoparticle Using Insulin Plant (Costus Pictus D. Don) and Investigation of Its Antimicrobial as Well as Anticancer Activities. *Adv. Nat. Sci. Nanosci. Nanotechnol.* 2018;9:015008. doi: 10.1088/2043-6254/aaa6f1. [CrossRef] [Google Scholar]

Swami K. D. And Bisht N.P.S., “Constituents of Ficus religiosa and Ficus infectoria and their biological activity”, J. Indian Chem. Soc., 1996, vol. 73 Nov, pp. 631.

Thema F.T., Manikandan E., Dhlamini M.S., Maaza M. Green Synthesis of ZnO Nanoparticles via Agathosma Betulina Natural Extract. *Mater. Lett.* 2015;161:124–127. doi: 10.1016/j.matlet.2015.08.052. [CrossRef] [Google Scholar]

van Vuuren S.F. Antimicrobial Activity of South African Medicinal Plants. *J. Ethnopharmacol.* 2008;119:462–472. doi: 10.1016/j.jep.2008.05.038. [PubMed] [CrossRef] [Google Scholar]

Vanlaveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B and Rokhum SL, 2021. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature. RCS advances. 5.

Wan F, Feng C, Luo K, Cui W, Xia Z, Cheng A. Effect of steam explosion on phenolics and antioxidant activity in plants: a review. *Trends Food Sci Technol.* 2022;124:13. doi: 10.1016/j.tifs.2022.04.003. [CrossRef] [Google Scholar]

Wang H, Wang Z, Yue R, Gao F, Ren R, Wei J, ... and Kong Z (2020) Functional group-rich hyperbranched magnetic material for simultaneous efficient removal of heavy metal ions from aqueous solution. J Hazard Mater 384:121288 [PubMed]

Weldegebrieal G.K. Synthesis Method, Antibacterial and Photocatalytic Activity of ZnO Nanoparticles for Azo Dyes in Wastewater Treatment: A Review. *Inorg. Chem. Commun.* 2020;120:108140. doi: 10.1016/j.inoche.2020.108140. [CrossRef] [Google Scholar]

Wen J, Fu W, Ding S, Zhang Y, Wang W. Pyrogallic acid modified nanoscale zero-valent iron efficiently removed Cr (VI) by improving adsorption and electron selectivity. *Chem Eng J.* 2022;443:136510. doi: 10.1016/j.cej.2022.136510. [CrossRef] [Google Scholar]

Xu J., Huang Y., Zhu S., Abbes N., Jing X., Zhang L. A Review of the Green Synthesis of ZnO Nanoparticles Using Plant Extracts and Their Prospects for Application in Antibacterial Textiles. *J. Eng. Fiber Fabr.* 2021;16 doi: 10.1177/15589250211046242. [CrossRef] [Google Scholar]

Xu Q, Li W, Ma L, Cao D, Owens G, Chen Z. Simultaneous removal of ammonia and phosphate using green synthesized iron oxide nanoparticles dispersed onto zeolite. *Sci Total*

*Environ.* 2020;703:135002. doi: 10.1016/j.scitotenv.2019.135002. [PubMed] [CrossRef] [Google Scholar]

Yadav P., Kant R and Kothiyal P., 2013. A revie on Acacia tortilis. International journal of pharmaceutical and phytopharmacological research. 3(2): 93-96.

Zhang, R., Li, J., Zhang, T. *et al.* Importance of integrating nanotechnology with pharmacology and physiology for innovative drug delivery and therapy – an illustration with firsthand examples. *Acta Pharmacol Sin* **39**, 825–844 (2018). https://doi.org/10.1038/aps.2018.33.